Calcium Infusion

A New Provocative Test for Insulinomas

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Calcium gluconate (10 mg Ca^{++}/kg) was administered intravenously over a 2-hour period to 16 adult patients who were evaluated for hypoglycemia. In nine of ten patients with benign or malignant insulinomas (eight proven at operation, and two with positive chemical tests and angiographic localization awaiting operation), significant hypoglycemia and hyperinsulinemia occurred within 60 to 90 minutes after the start of the calcium infusion. Serum proinsulin and C-peptide concentrations increased at the time of the calcium-induced hyperinsulinemia in several patients in whom these parameters were studied. The one individual who did not respond to the calcium infusion was found to have a benign insulinoma. His basal glucose/insulin ratio of 0.64 was the lowest of the insulinoma group and thus his failure to respond to calcium may indicate that his tumor was secreting maximally at the time of the infusion. Following successful removal of the insulinoma, calcium infusion did not result in changes in serum glucose or insulin concentrations (tested in five patients). In contrast, neither a patient with pathologically documented islet cell hyperplasia, five others with reactive, functional or drug-induced hypoglycemia, nor four healthy volunteers showed any changes in circulating glucose or insulin levels while receiving calcium intravenously. Calcium infusion is a safe, rapid and effective provocative test for the diagnosis of insulin-secreting, islet cell tumors of the pancreas.

THE DIFFERENTIATION of insulin-secreting tumors of the pancreas from other hypoglycemic states is often difficult and time-consuming. As early as 1935, Whipple and Frantz[14] wrote, ". . . there are many causes for hypoglycemia which are not due to lesions of the pancreas . . . these conditions should be ruled out clinically before exploratory operation is justifiable."

Calcium infusion has been utilized as an effective provocative test for the diagnosis of medullary carcinomas of the thyroid,[2] gastrinomas[10] and carcinoid tumors.[7] In each instance, a peptide or amine—calcitonin, gastrin or serotonin—is discharged from the tumor into the circulation. We have previously reported that the administration of calcium resulted in an increased serum concentration of insulin and proinsulin in one patient with an insulin-secreting tumor.[4] The purpose of this study was to determine the efficacy of calcium infusion as an aid in the diagnosis of hypoglycemic states with particular reference to insulinomas.

Materials and Methods

Sixteen adult patients with hypoglycemia who were admitted to the University of Chicago Hospitals or to the Michael Reese Hospital were studied. Each received a conventional series of diagnostic tests, including some or all of the following: a prolonged fast, an intravenous or oral glucose tolerance test and an intravenous tolbutamide test. Ten of these patients were found to have insulinomas (eight have been surgically removed, two await operation, but the presence of the tumor has been demonstrated by angiogram), one child had surgically proven islet cell hyperplasia, while five others were diagnosed as having reactive, functional or drug-induced hypoglycemia and were not operated upon.

In addition to the studies listed above, these 16 patients and four additional non-diabetic volunteers received an infusion of calcium gluconate, 10 mg Ca^{++}/kg in saline, over a two-hour period following a six-

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to eight-hour fast. The calcium infusion was repeated postoperatively in five of the patients in whom insulinomas were surgically removed.

Blood was drawn from a peripheral vein at regular intervals before and during the infusion. Plasma glucose was measured, within minutes, using a Beckman glucose analyzer and subsequently checked by an autoanalyzer technique[5] in the clinical laboratory. Serum calcium (atomic absorption spectrophotometry), and immunoreactive insulin[9] concentrations were also determined in each sample. In several selected individuals, serum proinsulin[13] and immunoreactive connecting peptide (C-peptide)[6] concentrations were determined. Proinsulin was separated from insulin by gel filtration and measured against a human proinsulin standard. Statistical analysis was determined using the Student's t-test.

Results

As a group, the ten insulinoma patients responded dramatically to the infusion of calcium (Fig. 1, left). The basal glucose level prior to infusion was 68 ± 7 mg/dl (mean ± S.E.). Plasma glucose fell markedly within 60 minutes of the start of the infusion and symptoms of hypoglycemia developed in many individuals. The lowest value of serum glucose, 31 ± 5 mg/dl ($p < 0.005$ compared to the basal level), occurred at 60 minutes after the start of the infusion. When patients became symptomatic, the calcium infusion was discontinued and intravenous glucose was administered with prompt reversal of all hypoglycemic effects. In these ten patients, serum insulin concentrations increased as well. Mean basal serum insulin value was 38 ± 8 microunits per milliliter (μU/ml). The peak value of insulin, 87 ± 28 μU/ml, occurred at 30 minutes after the start of the calcium infusion ($p < 0.05$). By 60 minutes, serum calcium concentrations had increased by more than 2 mg/dl ($p < 0.005$).

The maximum changes in each individual of both circulating glucose and insulin concentrations (left) and of the glucose:insulin (G/I) ratio (right) during the calcium infusion is shown in Figure 2. Nine of ten individuals who were studied demonstrated a clear decrease in plasma glucose and a rise in serum insulin levels as a result of the induced hypercalcemia. In addition, the glucose to insulin ratio decreased to less than 50% of the basal value in nine of ten patients. One individual, P.W., a 21-year-old man with a benign insulinoma, did not respond to the calcium infusion as did the others. Of interest is the fact that his basal values of glucose and insulin were 55 mg/dl and 94

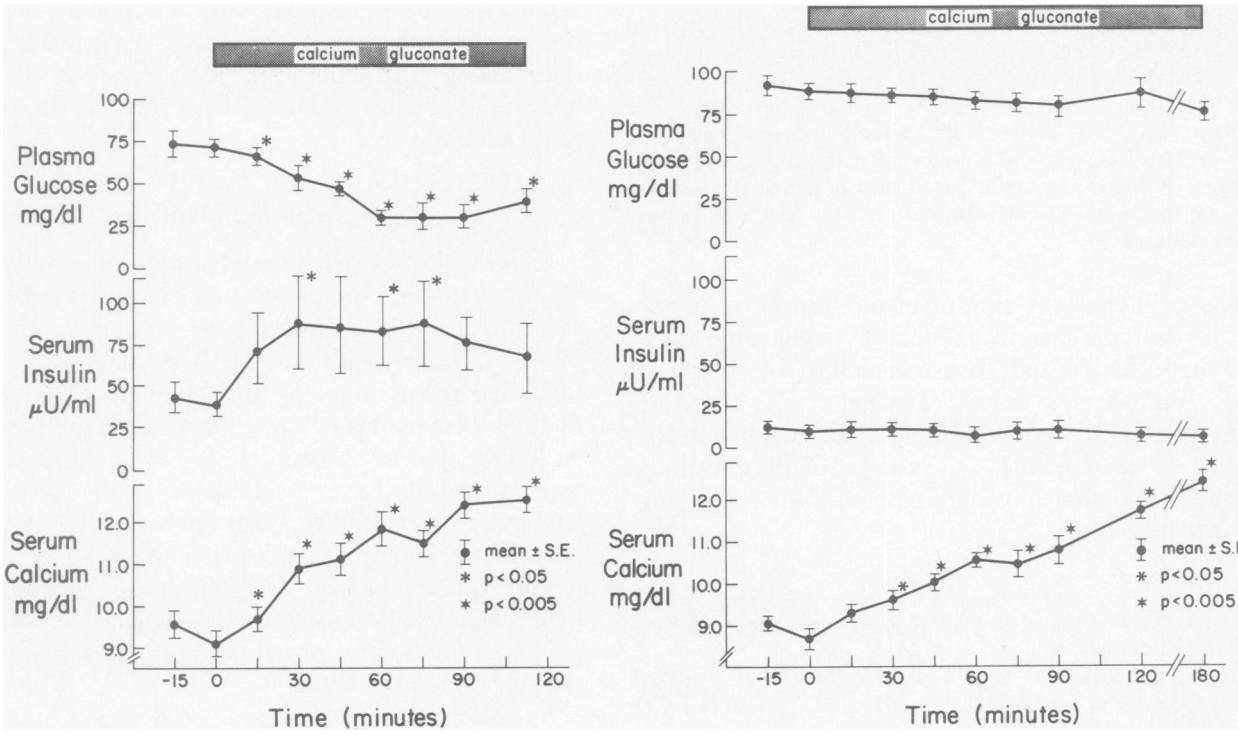

FIG. 1. The effect of calcium infusion (5 mg Ca^{++}/kg/hr in saline) in patients with insulin-secreting tumors of the pancreas. *Left*—Preoperative studies in ten patients. Marked hypoglycemia and hyperinsulinemia followed the start of the infusion. *Right*—Postoperative studies after successful removal of benign or malignant insulinomas in five of these individuals. Note that no changes in circulating glucose and insulin levels occurred during this three-hour test period.

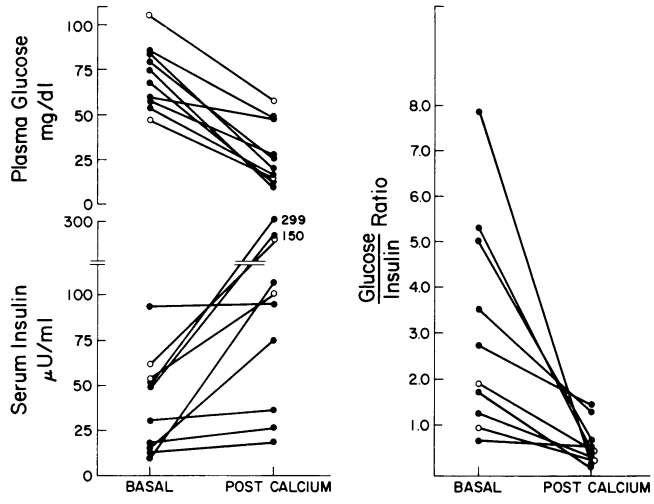

FIG. 2. Individual responses of ten patients with insulinomas to calcium infusion. Eight tumors have been surgically removed, ● —— ●; in two other patients ○ —— ○, insulinomas have been diagnosed by provocative tests and arteriographically localized but they await operation. *Left*—Maximum changes in plasma glucose and serum insulin concentrations during the calcium infusion. *Right*—Maximum changes in glucose: insulin ratio during induced hypercalcemia.

μU/ml, respectively. Thus, his basal glucose:insulin ratio was only 0.64 at the start of the infusion, the lowest of any of the insulinoma group. His G/I ratio decreased to 0.52 at two hours and 0.45 at three hours, a maximum decrease of only 30%. We consider this response to be a negative result, perhaps due to the fact that the tumor was secreting maximally at the time of the start of the calcium infusion.

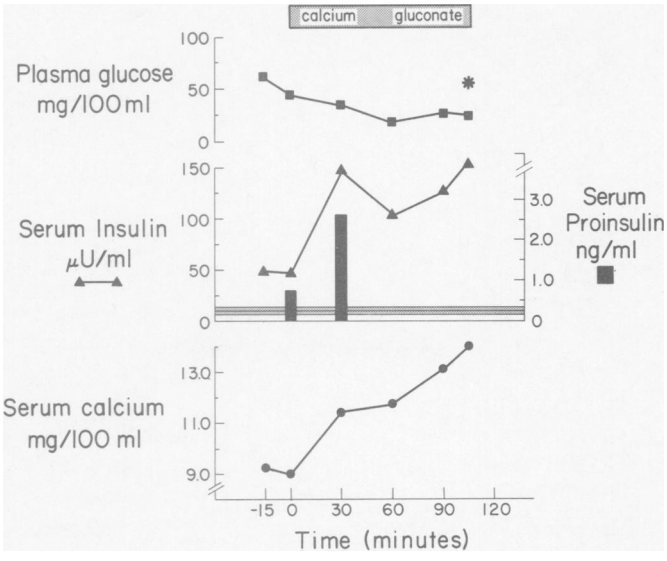

FIG. 3. In patient A.C., who was later proved to have a well-differentiated islet cell carcinoma, serum proinsulin levels, which were elevated in the basal state, increased approximately threefold at the time of maximum hyperinsulinemia induced by calcium. The hatched area represents the normal fasting value of circulating proinsulin (mean ± S.D.) as determined in 46 normoglycemic individuals. The calcium infusion was stopped after 105 minutes (*) because of the development of hypoglycemic symptoms.

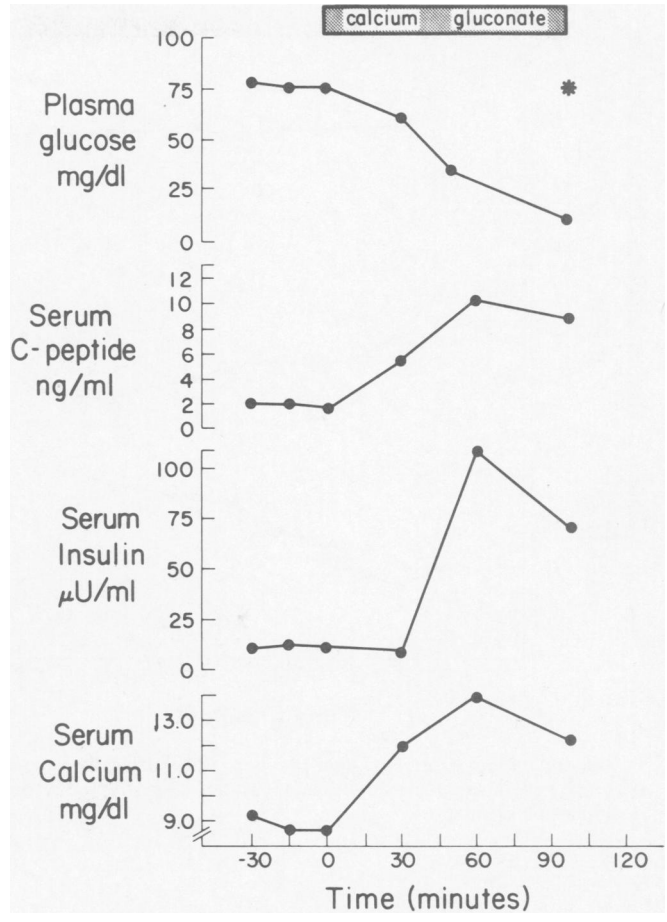

FIG. 4. In patient L.C., from whom a benign insulinoma was later removed, calcium infusion resulted in a five-fold increase in circulating C-peptide levels at the time of maximal hyperinsulinemia. The infusion was stopped (*) because he developed hypoglycemic symptoms.

In two individuals, A.C. and L.C., serum proinsulin and C-peptide levels were measured as well. Calcium infusion resulted not only in hyperinsulinemia, but also in a rise in these parameters (Figs. 3 and 4).

The calcium infusion was repeated in five of the insulinoma patients six months to two years after successful removal of their tumors (Fig. 1, right). Each individual was normoglycemic, had a normal basal insulin concentration and manifested no evidence of recurrence of tumor at the time of testing. In marked contrast to their preoperative studies, no significant changes in plasma glucose or serum insulin levels, and no untoward symptoms occurred during the calcium administration.

One child with surgically proven islet-cell hyperplasia manifested no significant changes in either plasma or serum insulin values while receiving the calcium infusion (Fig. 5).

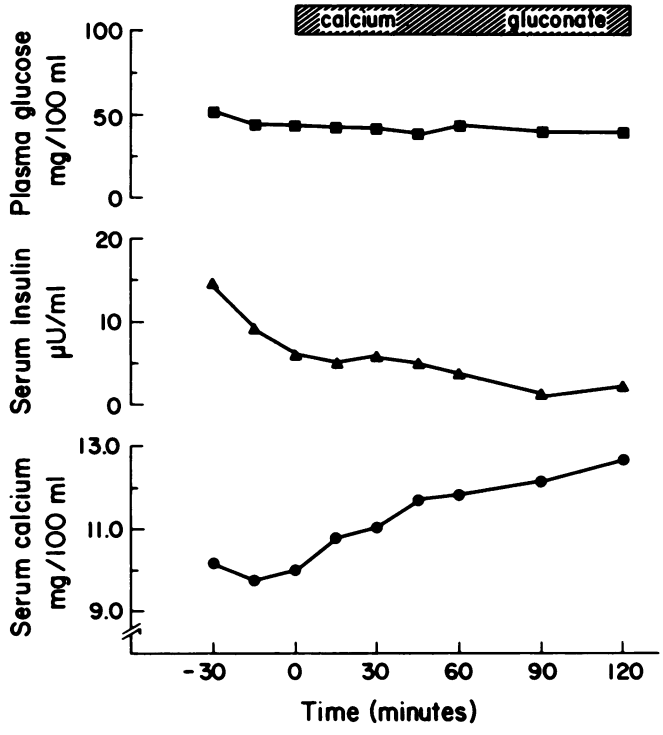

FIG. 5. In patient C.Z., a 7-year-old boy with surgically proved islet cell hyperplasia, calcium infusion resulted in no hypoglycemia or hyperinsulinemia.

Finally, five individuals with a diagnosis of either reactive, functional or drug-induced hypoglycemia (Fig. 6, left) and four normoglycemic volunteers (Fig. 6, right) received a calcium infusion. None experienced symptoms of hypoglycemia and circulating glucose and insulin concentrations did not change significantly from baseline values.

Discussion

The differentiation of insulin-secreting tumors from other causes of hypoglycemia may sometimes be difficult and require time-consuming tests. A simple test which could facilitate the diagnosis of islet-cell tumors would be beneficial. We have found calcium infusion to be useful in this regard. It is simple, rapid and safe when conducted in a setting in which plasma glucose values can be rapidly monitored. We perform this infusion in our Clinical Research Center with a physician in attendance. Plasma glucose concentrations were measured within several minutes of obtaining a blood sample using a Beckman glucose analyzer. Hypoglycemic symptoms induced by the calcium were rapidly reversed with intravenous glucose and no other untoward symptoms related to the mild hypercalcemia have been noted.

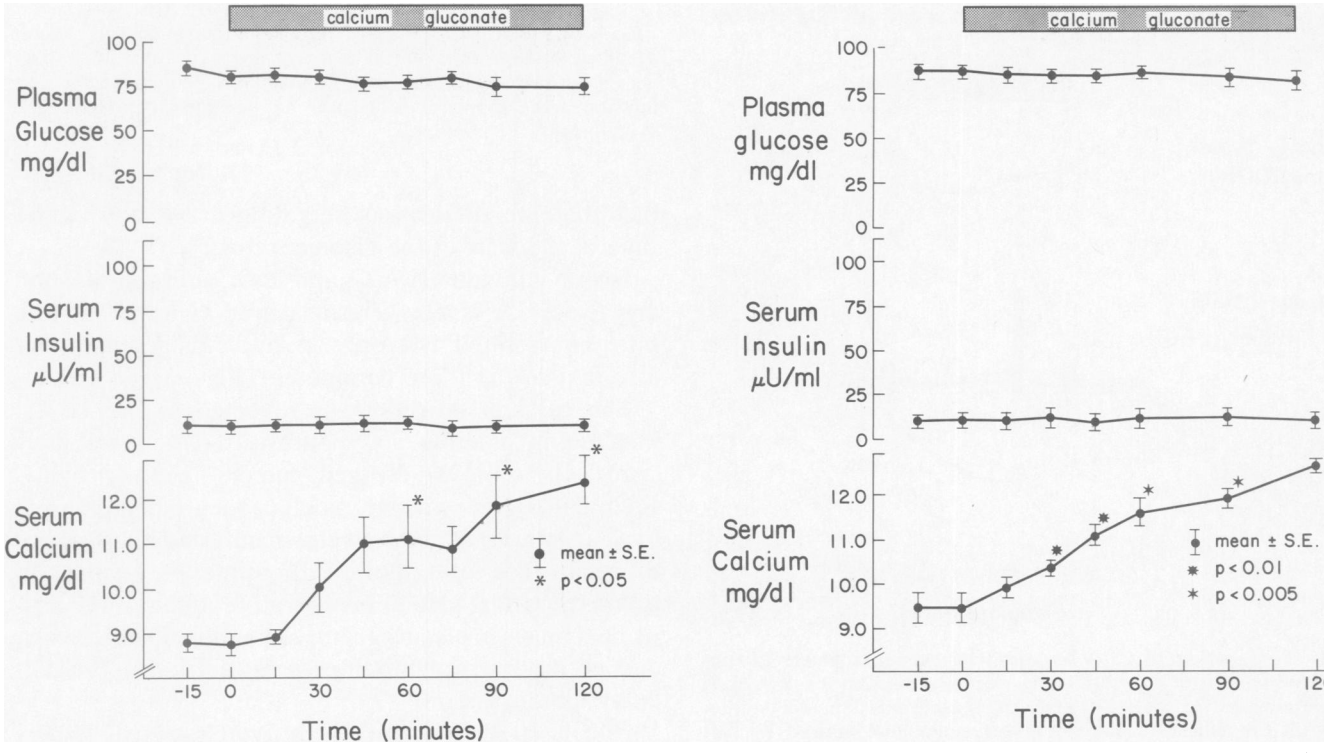

FIG. 6. In five individuals with reactive, functional or drug-induced hypoglycemia (left) and in four other normoglycemic volunteers (right), calcium infusion resulted in *no* significant changes in plasma glucose or serum insulin concentrations.

TABLE 1. *Comparison of Results of Prolonged Fasting and Calcium Infusion Tests in Ten Patients with Insulinomas*

	Plasma Glucose (mg/dl)	Serum Insulin (μU/ml)	G/I Ratio	Duration of Test
Fasting Test				
Initial values*	73.5 ± 7.5	35.1 ± 7.4	3.4 ± 0.8	
Final values†	28.1 ± 2.9	25.0 ± 6.3	1.4 ± 0.3	19.7 ± 3.4 hours
Calcium Infusion Test				
Initial values*	72.1 ± 5.7	39.4 ± 8.5	3.0 ± 0.7	
Final values†	28.7 ± 5.2	90.4 ± 21.1‡	0.5 ± 0.5§	90 ± 11.8 minutes

* Following a six–eight hour fasting period. Each value represents the mean ± S.E. of ten patients.

† The tests were terminated when symptoms of hypoglycemia occurred.

‡ $p < 0.05$, when compared to values of serum insulin levels at the end of the fasting test.

§ $p < 0.005$, when compared to values of G/I ratio after prolonged fasting.

Since our original report of one patient in whom calcium infusion was useful in diagnosing an insulin-secreting tumor,[4] we and others[12] have continued to study the efficacy of this test. Our results demonstrate that in nine of ten patients with insulinomas (five benign, three malignant, two awaiting operation), marked hypoglycemia and hyperinsulinemia occurred within 60–90 minutes of initiating the infusion. Calcium infusion also induces a release of proinsulin and C-peptide from insulin-secreting tumors. Neither patients with reactive or drug-induced hypoglycemia, normal non-diabetic volunteers, nor one child with islet-cell hyperplasia developed hypoglycemia or an elevated serum insulin concentration during or after calcium infusion. However, this test gave a negative result in one patient with a benign insulinoma, perhaps due to the fact that his tumor was secreting maximally before the calcium was administered.

Our original report has recently been confirmed by Roy et al.[12] In each of four patients with either benign or malignant insulinomas whom they studied, calcium infusion resulted in marked hypoglycemia and hyperinsulinemia. Furthermore, they demonstrated that these effects could be partially blocked by epinephrine[11] and completely blocked by an infusion of diazoxide.[3] Both of these agents are known to inhibit insulin secretion from β-cells. Of particular interest in this regard is the fact that in *in vitro* studies diazoxide inhibition of insulin secretion from rat islet cells is associated with a net efflux of calcium into the incubation medium.[8]

Thus, the cumulative data of Roy et al.[12] and our present studies demonstrate no false positive tests and only one false negative study in 14 consecutive insulinoma patients who were studied by calcium infusion. We have found this test to be of diagnostic value in several patients in whom other studies involving the stimulation of insulin secretion were equivocal. Furthermore, thus far, all patients with functional or reactive hypoglycemia proven by other diagnostic studies have had negative calcium infusion tests. A negative test also occurred in the one child with islet cell hyperplasia. Clearly more children with proven islet cell hyperplasia or nesidioblastosis should be evaluated before the value of this test in differentiating these conditions from insulinomas is known.

Finally, when the results obtained with preoperative calcium infusion in 10 patients with insulinomas were compared with those obtained following prolonged fasting (Table 1), one can see that plasma glucose values were comparable at the end of both tests. However, serum insulin levels were statistically higher and G/I ratios were statistically lower at the end of the calcium infusion test. The duration of fasting until symptoms occurred was 11.5 to 48 hours (19.7 ± 3.4, mean ± S.E.), while the calcium infusion test took two hours or less. Prolonged fasting is still the standard to which all other tests for insulinomas should be compared; however, the calcium infusion test is less time consuming and has a very high degree of accuracy.

On the basis of these studies, we now consider the calcium infusion test to be an important procedure in the preoperative selection of hypoglycemic patients for operation.

Acknowledgments

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Discussion

Dr. Don R. Miller (Irvine, California): The intrigues of diagnosis and treatment of insulinoma continue to challenge the surgeon. This is another interesting and apparently highly accurate method of preoperative diagnosis.

I would like to digress to the operative diagnosis of insulinoma. The preoperative localization of small and multiple tumors has been greatly aided by the arteriographic demonstration of a blush, or subselective venous, sampling but even with these sophisticated methods the surgeon may be misled as to the correct operation to execute at the operating table. This is particularly true of occult and multiple lesions.

(slide) The use of the artificial pancreas, appropriately called the beta cell, provides computerized constant digital readout of blood sugar (slide). (This patient had 37 mg/dl at the start of the operation.) and provides a constant selected blood sugar level by surveillance and appropriate, selective glucose or insulin administration. This is another elegant and expensive tool to assist the surgeon in the operating room.

In collaboration with Drs. Tanner and Valenta in a recent instance in a young woman with Whipple's triad who had been treated ineffectively for 15 years for epilepsy (slide), this preoperative venous-phase arteriogram showed a blush in the midbody of the pancreas, and also a small, circumscribed opacity perhaps an accessory spleen, localized outside of the pancreas.

(slide) Only a 1.5 cm mass in the extreme tail of the gland, not in either anticipated location, was found at operation and was removed. Even prior to receiving the frozen section pathologic report confirming this to be an insulinoma (slide), the beta cell had shown a tenfold reduction in glucose requirement, and a noticeable progressive rise in the blood sugar level from 80 mg/dl, which was the programmed level, to 96 mg/dl. This rise is shown here. It assured removal of the source of excessive insulin had been accomplished and probably prevented a more radical removal of pancreatic tissue that would have been done if we had relied only on the arteriogram.

The postoperative glucose tolerance tests and glucose determinations in the short postoperative phase have been within the normal range.

This is an expensive tool for internists, but its cost-effectiveness is improved by this surgical application.

Dr. Donald S. Gann (Baltimore, Maryland): I believe that the calcium infusion offers a dramatic improvement in our diagnostic capabilities, one that offers particularly a kind of safety that has been difficult to achieve in this group of patients.

I think all of us who have attempted to work with this class of patient have experienced the patient who has a seizure in the middle of the night with inadequate observation. The use the calcium infusion prevents that occurrence by letting the patient become hypoglycemic rapidly at a time when observation is maximal, rather than when it is going to be minimal. Thus, it offers a real improvement in patient safety.

I think it also offers a major improvement in speed of diagnosis, since the monitoring of glucose clearly gives the person conducting the test the answer well before the endocrine laboratory can provide the hormonal confirmation.

I would like to ask two questions, though. First, I wonder if Dr. Kaplan can explain why calcium infusion does not evoke release of insulin in patients with hyperplasia of the beta cells. One would think that there would be adequate storage of insulin in such patients, even though it is more diffusely located in the gland.

Second, I wonder whether in a laboratory which can measure the C-peptide it is necessary to use a calcium infusion test, or whether simple measurement of the C-peptide in the resting state might be sufficient to make the diagnosis.

Dr. Samuel A. Wells (Durham, North Carolina): My comments are related primarily to the method of infusing calcium.

We have had experience with a similar type of tumor, that is, medullary thyroid carcinoma (MTC). Embryologically, the MTC cells, like the islet cells, are derived from the neural crest. The MTC cells secrete calcitonin, which serves as a plasma marker for this tumor. Early in the study of patients having MTC, it was found that a calcium infusion (15 mg/kg/4 hr) caused an increased secretion of calcitonin, thus allowing the diagnosis of MTC to be established, even though it was not apparent clinically. It was soon appreciated that a shorter calcium infusion (2 mg/kg/l min) produced a greater rise in calcitonin level more quickly in patients with MTC than did the four hour calcium infusion.

Dr. Kaplan's first plasma sample was at 15 minutes. I wondered if he has used the shorter calcium infusion test and if he has ever sampled at an earlier period after the calcium infusion. The short-term calcium infusion test would probably be safer and cause a higher peak level of insulin than would the long-term infusion.

Dr. James C. Thompson (Galveston, Texas): The mechanism for release of an active agent by calcium has been studied by several people, and Gardner has shown that in pancreatic exocrine cells it is probably caused by activation of cyclic GMP as a separate messenger.

The calcium test for gastrin release from Zollinger-Ellison tumors and for release of calcitonin from MTC tumors depends upon